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COMPARISON OF GEOMETRIC MORPHOMETRIC CHARACTRISTICS OF BODY AND GENITAL PLATE OF *EURYGASTER INTEGRICEPS* FEMALES FOR DISCRIMINATION OF POPULATIONS

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ABSTRACT

Many studies about morphology have been conducted in the field of entomology that their aims are clarification the relationship in closely related taxa and identification different population within and between species. This study was conducted to determine differences in female body and genitalia plate of the *Eurygaster integriceps* (Scutelleridae) by looking at variation in the shapes using the method of geometric morphometrics. This research was conducted in Iran in 2013. Cross-validation tests based on discriminant functions (DFA) showed that genitalia plate of females can better discriminate populations (54%) than body of females (50.25%). Multivariate analysis on genital plates identified significant differences between populations from different areas ($F=2.1$, $p=0.002$). Additionally, multivariate analysis on bodies identified no significant differences between populations ($F=1.15$, $p=0.3$). Allometry testing on genital plates was identified a significant difference between the size (centroid size) of the genital plates and 28 shape variables (Wilks' lambda=2.47, $p=0.0001$).

KEYWORDS: Genital Plates, Geometric Morphometrics, *Eurygaster integriceps*, Allometry

INTRODUCTION

The wheat bug, *Eurygaster integriceps* (Hemiptera: Scutelleridae), is one of the major wheat pests causing high yield loss in terms of wheat production in the Iran. Morphometric differentiations may explain biogeographic distinctions among populations of a given species. Among different species of wheat pests that exist in Iran, *Eurygaster integriceps* is the most widespread species and was conceived to be studied using geometric morphometrics analysis.

Species delimitation using phenotypic markers is widespread in the scientific literature. As a rule, anything observed on organisms is potentially useful in identifying species boundaries, provided that the extent to which the features concerned is representative of the organism as a whole (Rojas, 1992; Zink and McKitrick, 1995; Duffy, 1996; Turner, 1999; de Queiroz, 2005). This happens when variation in a phenotypic trait is caused by underlying genetic variation and not due to plastic changes in response to environmental changes. Morphology is the most generally used form of phenotypic marker for systematic purposes, but others exist as well.

A relatively recent method in quantifying biological form is the introduction of geometric morphometrics (GM). There are two methods in describing the form of an organism through geometric morphometric; first, by landmark based analysis that uses a set of landmarks to describe the object or specimen; second, by outline based analysis which extract the

margin around the specimen. GM analyzes biological form in such a way that will preserve the physical integrity of the form (Richtsmeier *et al* 2002). It allows better comparison of the shapes of different organisms and would no longer rely on word descriptions that usually encounter problem of being interpreted differently by scientists (Adams *et al* 2004).

Instead of distances and angles, geometric morphometric uses the coordinates of points called landmarks. The landmarks are superimposed by translation, scaling, and rotation. After superposition, the landmark configurations differ only in shape and can be analyzed by multivariate statistical methods (Zelditch et al., 2004). In wheat bug, geometric morphometric analyses of body and genitalia plate have provided many new insights into characters and identification of populations or lineages (Cruz *et al*., 2011).

Since there is no published information on geometric morphometric of *E. integriceps*, to fill this gap in information evaluations of *E. integriceps* was conducted. The aim of this study was comparison of variations in the body and genitalia plate of populations in different areas using a geometric morphometric method. Questions we addressed with this method were whether geographically isolating landforms such as mountains, valleys, or other natural obstacles affect morphological characteristics; whether this geometric morphometric method can discriminate populations in ten areas based on the bodies and genital plates; and which body and genitalia better discriminates area populations.

MATERIALS AND METHODS

The colonies were sampled from ten areas in different geographic regions of Iran (Sanandaj: 35° 25´ N, 46° 57´ E; Bijar: 35° 53´ N, 47° 32´ E; Dehgolan: 35° 15´ N, 47° 25´ E; Irankhah: 36° 08´ N, 46° 44´ E; Ghorveh: 35° 07´ N, 47° 48´ E; Kamyaran: 34° 47´ N, 46° 56´ E; Marivan: 35° 31´ N, 46° 10´ E; Tazeabad: 35° 41´ N, 46° 59´ E; Divandareh: 35° 50´ N, 47° 04´ E; Saghez: 36° 13´ N, 46° 22´ E) (Table 1). Sampling was conducted from wheat fields by sweeping. 40 samples were selected from collected samples from every wheat field. Samples were mounted on plots by Shellac gel in suitable positions. Digital photos were taken from mounted bodies and genital plates using a DP12 camera and a SZX12 OLYMPUS stereomicroscope. A total of 100 closely connected points were made along the outline of the body and genital platein order to capture the general shape using a digitizing program tpsDig version 2.16 (Rohlf 2008a) (figure 1 & 2). Then, the tps curve was converted into landmark points (XY coordinates) that would serve as raw data for outline analysis.

Eighteen landmarks on female bodies and sixteen landmarks on female genital plates were digitized on photos for their capacity to define the major elements of shape and for their reliability as homologous structure by tpsDig software (figure 1 & 2). A Generalized Procrustes analysis (GPA) was performed to superimpose the landmark configurations using least-squares estimates for translation and rotation parameters (Adams *et al* 2004). Next, digitized landmark outputs were analyzed by tpsRelw, tpsReg (Rohlf, 2010), and NtSys Pc. 2. Finally, samples of ten areas were classified with discriminant function analysis (DFA) by SPSS ver. 18. All non-shape variations of these landmarks such as orientation (or rotation), scale, and size were removed. A multivariate analysis of variance (MANOVA) was carried out on the landmark data to compare *E. integriceps* populations.

RESULTS

Variation averages of outline landmarks of body and genital plate showed that outline variation of genital plate was more than outline variation of body between populations. Additionally, Asymmetry in the shapes of left and right sides in the genital plates were observed among female populations (figure 4). The relative warp analysis (RWA) in the bodies

and genitalia plates of *Eurygaster integriceps* demonstrated variations in the outlines. The Three significant relative warps jointly accounted for 74.7% and 87.29% of the variation in the shape of bodies and genital plates respectively. Multivariate analysis of variance (MANOVA) on relative warp scores showed that there was no significant variation in the bodies between populations (F=1.15, P=0.3). Moreover, There was significant variation in shapes of genital plates between populations (F=2.1, P=0.002).

Variance of coordinates of 18 landmarks on bodies and 16 landmarks on genital plates as well as coordinates of landmarks of average shape were evaluated. At the body, the eighteenth and seventeenth landmarks, the posterior of body, had the maximum variation (S^2=0.00019 and 0.00018 respectively) in collected populations from ten areas of Iran; Furthermore, the third and forth landmarks, the junction of anterior of eyes to head, had the minimum variation. At the genital plates, the sixteenth landmark, the posterior of genital plates (S^2=0.00036), had the maximum variation; landmarks 2 and 3 had the next greatest variations. Eighth and eleventh landmarks, center of genital plates, had the minimum variation in collected populations from ten areas (figure 3 & 4).

Allometry testing on bodies and genital plates was performed. The results identified a significant difference between the size (centroid size) of the bodies and 32 shape variables (Wilks' lambda=0.68, p=0.000); therefore, the shape variations of the bodies were not uniform. Then, with the increasing of size of the bodies, body shape changed. In addition, there was allometry in genital plates between centroid sizes and 28 shape variables (Wilks' lambda=0.79, p=0.0001).

Centroid sizes of bodies and genital plates were compared in different geographical areas of Iran. Results showed a significant difference in centroid size of bodies (F=2.14, p=0.02). Bodies of *E. integriceps* populations in Ghorve area had maximum centroid size (C=1575.4), but there was no significance difference with Dehgolan and saghez area. Additionally, the bodies of the Marivan population had the minimum centroid size (C=1548.7). Analysis of centroid sizes of genital plates revealed significant differences among the ten areas (F=3.1, p=0.001). The *E.integriceps* population of Divandareh had the maximum centroid size (C=1569.6) but did not differ significantly from the populations of Tazeabad, Saghez, Irankhah, Bijar, dehgolan, Marivan and Ghorveh. The genital plates of the *E. integriceps* population of Kamyaran had the minimum centroid size (C=1502.2).

Populations of ten zones based on shape variables of bodies and genital plates were classified by DFA. Cross-validation tests based on discriminant functions of bodies correctly classified 50.25% of the colonies (table 2). Statistical analysis results for 32 shape variables of the bodies by DFA showed that the *E. integriceps* population of Bijar was almost separated (67.5%) from other areas (figure 5). Cross-validation tests based on discriminant functions of genital plates correctly classified 54% of the colonies (table 3). Statistical analysis results for 28 shape variables of the genital plates by DFA showed that the *E. integriceps* population of Dehgolan was almost separated (76.7%) from other areas (figure 5).

Relations of geographical populations were evaluated using the UPGMA (unweighted pair group method with arithmetic means) method. The cladogram resulting from the UPGMA cluster analysis of the bodies showed that the two populations of Marivan and Tazeabad were similar completly (figure 6). Cluster analyses divided populations into three main groups. The first group included the populations of Sanandaj, Irankhah and Kamyaran. The second group included three populations of Ghorve, Marivan and Tazeabad, and a third group contained Bijar and Dehgolan. This cluster was differentiated Saghez population completely form another area populations. Cluster analyses of the genital plates revealed

relatively different results (figure 7), so Divandareh and Saghez populations were similar completly. Additionally, population of Kamyaran was differentiated completely form another area populations.

DISCUSSIONS

Morphometric identification techniques, which have improved considerably thanks to new computational techniques, have also improved in practicality because they require little technical knowledge or specialized equipment (Francoy et al., 2008). Standard morphometrics has long been applied to discriminate honeybee subspecies, but such studies take considerable time to complete (Francoy et al., 2008). Geometric morphometric methods are more practical and easier and accomplished in a much shorter time because all procedures are based on computer-assisted technology (Zelditch et al., 2004).

Our results showed that *E. integriceps* population of Bijar was almost separated (67.5%) from other area populations. Bijar area was surrounded and isolated by mountain ranges and this area is situated in highland so it's famous to "roof of Iran".Three factors likely drive shape: (1) environmental pressures such as latitude (Alpatov, 1929), altitude (Verma et al., 1994; Hepburn et al., 2000), and climate (Hepburn et al., 2001; Radloff et al., 2005a,b; Tan et al., 2008); (2) sexual selection (Radloff et al., 2003a); and (3) abiotic factors such as temperature (Soose, 1954) and season (Mattu & Verma, 1984). Oyerinde et al. (2012) confirmed that a number of factors can affect taxonomy, especially agro-ecological zone factors.

Genital plate multivariate results showed that there was significant difference between *E. integriceps* populations of different areas. Additionally centroid sizes (size) of area populations were different. Bryantseva (1973) evaluated morphometric differences of *E. integriceps* populations. He showed that there was significant difference between three populations. Also, Sarafrazi (2001) identified seven population groups of *E. integriceps* in Iran. The possibility of morphometric distinctions between two populations of *Eurygaster maura* L. collected from the northeast (Golestan province) and the northwest (Azarbayejan province) of Iran was examined using principal component analysis over 25 body measurements (eight absolute and 17 proportional, separately). The northeast individuals were relatively larger and darker in colour than those of the northwest (Mohaghegh, 2005). The landmark-based geometric morphometric approach of relative warps (RW) was used to determine the population structures of the rice black bugs (RBB), *Scotinophara coarctata*, from the Philippines and in one site in Malaysia based on the shapes of the head and pronotum. The results suggested that the existence of morphological differences in the populations of RBB may indicate possible genetic differentiation (Torres et al., 2010).

Cross-validation tests based on discriminant functions (DFA) showed that genital plates of females can better discriminate populations (54%) than body of females (50.25%). Geographic variation in genital characteristics is an issue of both evolutionary and systematic importance. Genital characteristics in taxonomy indicate their value in species delimitation and they show great divergence between species. Genital plates also plays important role in species speciation and reproduction (Mutanen, 2006). Jansson and Pajunen (1978) conducted morphometric comparison of geographically isolated populations of *Arctocorisa carinata* (Hemiptera, corixidae). They showed that heterogeneity of the ecological grouping was further supported by multivariate analysis of the genital characters. Variation between the populations showed an obvious geographic pattern. Grazia et al., (2008) described *Chloropepla paveli* sp. nov. and *C. stysi* sp. nov., from Brazil based on the morphology of genitalia for both sexes. Cruz et al., (2012) evaluated the effect of rice varieties on

shape variation of genital plates of rice black bugs (RBB), *Scotinophara coarctata*. Results showed that rice types or genotypes affect on shape of genital plate.

CONCLUSIONS

Our results showed that variation of genital plate was more than variation of body between populations. Moreover, Cross-validation tests based on discriminant functions (DFA) showed that genital plates of females can better discriminate populations (54%) than body of females (50.25%). Moreover, multivariate analysis showed that there was significant difference between shapes of genital plate but bodies showed no significant difference between populations. Therefore, genital plate is more useful for discrimination of population of different geographical areas.

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APPENDICES

Figure 1: Eighteen Landmarks on Body (a) and Body Outline (b) of *Erugaster integriceps*

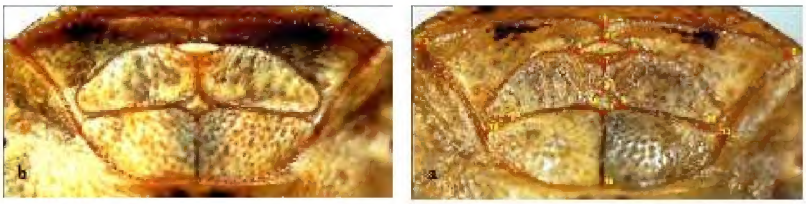

Figure 2: Sixteen Landmarks on Genital Plate (a) and Genital Plate Outline (b) of *Erugaster integriceps*

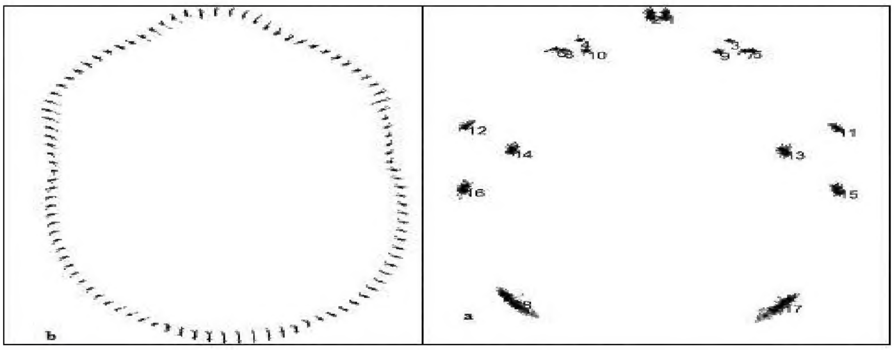

Figure 3: Average of Schematic Variations of 18 Landmarks on the Body (a) and Body Outline b) of *Eurygaster integriceps*

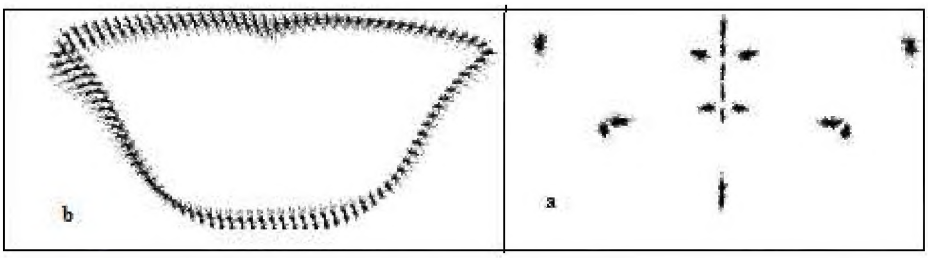

Figure 4: Average of Schematic Variations of 16 Landmarks on the Genital Plate (a) and Genital Plate Outline (b) of *Eurygaster integriceps*

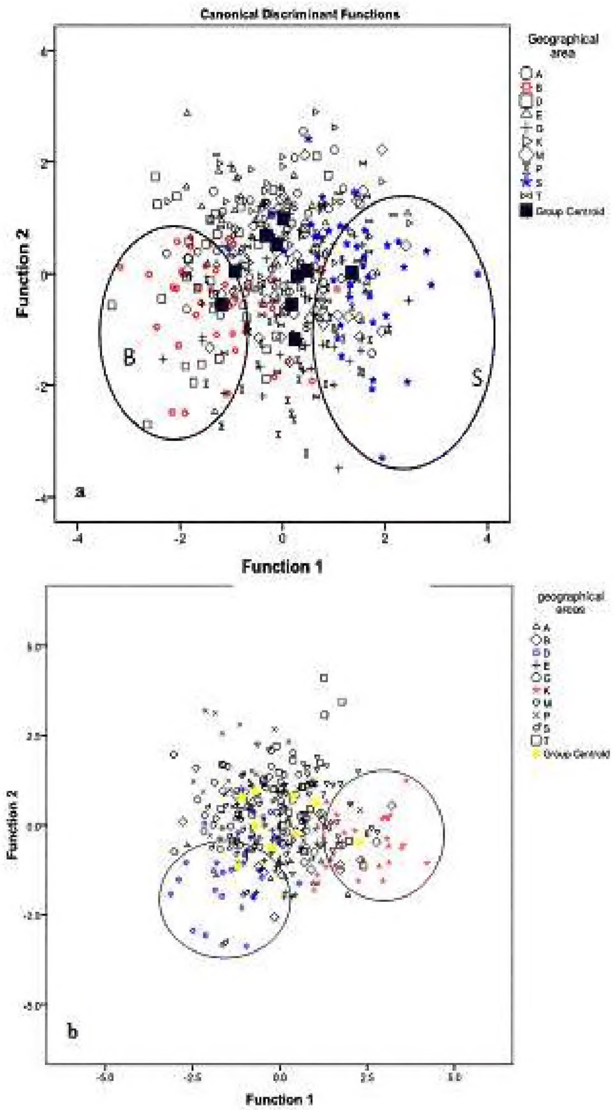

Figure 5: Scatter Plot of Discriminant Analysis in Bodies (a) and Genital Plates (b) of *Eurygaster integriceps* in Different Geographical Areas

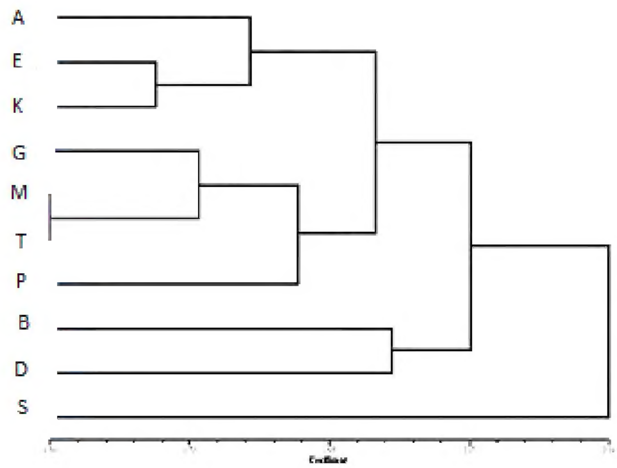

Figure 6: Dendrogram Resulting from a UPGMA Cluster Analysis of Samples from Geographical Populations Using Data from the Body

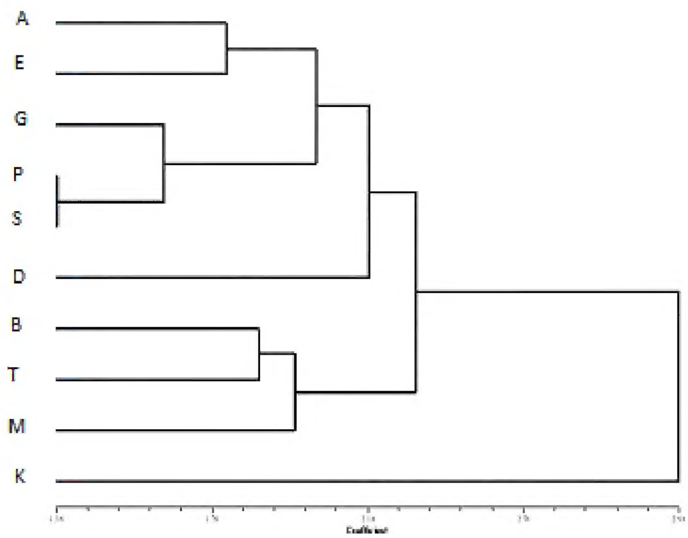

Figure 7: Dendrogram Resulting from a UPGMA Cluster Analysis of Samples from Geographical Populations Using Data from the Genital Plate

Table 1: List and Code of Collecting Sites of *Eurygaster integriceps* in Iran

Population	Body Genital Plate			
	Code		Code	Sample Number
Sanandaj	A	40	a	30
Bijar	B	40	b	30
Dehgolan	D	40	d	30
Irankhah	E	40	e	30
Ghorve	G	40	g	30
Kamyaran	K	40	k	30
Marivan	M	40	m	30
Tazeabad	T	40	t	30
Divandareh	P	40	p	30
Saghez	S	40	s	30

Table 2: Summary of the Colony Assignments of Body with Respect to Regions Based on Geometric Morphometrics. Percent Classifications are in Parentheses; N Denotes the Number of Specimens

Area	A	B	D	E	G	K	M	P	S	T
A	22 (55)	-	-	-	-	-	-	-	-	-
B	-	27 (67.5)	-	-	-	-	-	-	-	-
D	-	-	19 (47.5)	-	-	-	-	-	-	-
E	-	-	-	-	-	-	-	-	-	
G	-	-	-	-	18 (45)	-	-	-	-	-
K	-	-	-	-	-	19 (47)	-	-	-	-
M	-	-	-	-	-	-	16 (40)	-	-	-
P	-	-	-	-	-	-	-	20 (50)	-	-
S	-	-	-	-	-	-	-	-	26 (65)	-
T	-	-	-	-	-	-	-	-		15 (37.5)

Table 3: Summary of the Colony Assignments of Genital Plate with Respect to Regions Based on Geometric Morphometrics. Percent Classifications are in Parentheses; N Denotes the Number of Specimens

Area	A	B	D	E	G	K	M	P	S	T
A	14 (46.7)	-	-	-	-	-	-	-	-	-
B	-	11 (36.7)	-	-	-	-	-	-	-	-
D	-	-	23 (76.7)	-	-	-	-	-	-	-
E	-	-	-	15 (50)	-	-	-	-	-	-
G	-	-	-	-	15 (50)	-	-	-	-	-
K	-	-	-	-	-	22 (73)	-	-	-	-
M	-	-	-	-	-	-	21 (70)	-	-	-
P	-	-	-	-	-	-	-	14(46.7)	-	-
S	-	-	-	-	-	-	-	-	10 (33.3)	-
T	-	-	-	-	-	-	-	-		17 (56.7)